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**Abstract:** Despite intensified salvage treatments, children with relapsed/refractory acute myeloid leukemia (AML) have poor survival. We evaluated gemtuzumab ozogamicin (CD33-targeted drug) used on a compassionate basis in patients diagnosed from 1995 until 2014 within Acute Myeloid Leukemia Berlin-Frankfurt-Münster studies, and identified 76 patients (<18 years) with highly-advanced and pre-treated AML [refractory acute myeloid leukemia (n=10), AML refractory to relapse (1 early: n=41; 1 late: n=10; 2 or more: n=10), and secondary AML (n=5)]. At doses of 2.5-10 mg/m, gemtuzumab ozogamicin was administered in 1-4 cycles as single agent (47%), combined with cytarabine (47%), or others (6%). Most common grade 3/4 adverse events were infections or febrile neutropenia (78% of severe adverse events), infusion-related immunological reactions (6%), and gastrointestinal symptoms (5%). Three patients experienced veno-occlusive disease (one fatal due to exacerbation of a pre-existing cardiomyopathy). Sixty-four percent received subsequent hematopoietic stem cell transplantation. Probability of 4-year overall survival was  $18 \pm 5\%$  in all,  $27 \pm 7\%$  in patients with and 0% in patients without hematopoietic stem cell transplantation ( $<0.0001$ ). Administration of gemtuzumab ozogamicin on a patient-specific, compassionate use basis was frequently considered in our study group and proved to be effective for bridging children with very advanced AML to hematopoietic stem cell transplantation. Uniform prospective studies for these patients are urgently needed.

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# Gemtuzumab ozogamicin in children with relapsed or refractory acute myeloid leukemia: a report by Berlin-Frankfurt-Münster study group

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## ABSTRACT

Despite intensified salvage treatments, children with relapsed/refractory acute myeloid leukemia (AML) have poor survival. We evaluated gemtuzumab ozogamicin (CD33-targeted drug) used on a compassionate basis in patients diagnosed from 1995 until 2014 within Acute Myeloid Leukemia Berlin-Frankfurt-Münster studies, and identified 76 patients (<18 years) with highly-advanced and pre-treated AML [refractory *de novo* acute myeloid leukemia (n=10), *de novo* AML refractory to relapse (1<sup>st</sup> early: n=41; 1<sup>st</sup> late: n=10; 2<sup>nd</sup> or more: n=10), and secondary AML (n=5)]. At doses of 2.5-10 mg/m<sup>2</sup>, gemtuzumab ozogamicin was administered in 1-4 cycles as single agent (47%), combined with cytarabine (47%), or others (6%). Most common grade 3/4 adverse events were infections or febrile neutropenia (78% of severe adverse events), infusion-related immunological reactions (6%), and gastrointestinal symptoms (5%). Three patients experienced veno-occlusive disease (one fatal due to exacerbation of a pre-existing cardiomyopathy). Sixty-four percent received subsequent hematopoietic stem cell transplantation. Probability of 4-year overall survival was 18±5% in all, 27±7% in patients with and 0% in patients without hematopoietic stem cell transplantation (*P*<0.0001). Administration of gemtuzumab ozogamicin on a patient-specific, compassionate use basis was frequently considered in our study group and proved to be effective for bridging children with very advanced AML to hematopoietic stem cell transplantation. Uniform prospective studies for these patients are urgently needed.

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## Introduction

Treatment of acute myeloid leukemia (AML) in children has improved remarkably during the past decades; however, pediatric patients with relapsed and refractory AML still have poor outcomes.<sup>1-6</sup> These outcomes rely on disease-dependent characteristics, such as initial cytogenetics, in addition to response-to-therapy-related factors like the interval between initial diagnosis and relapse.<sup>7-10</sup> Considering poor outcome and high toxicity of current salvage therapies, new targeted molecular treatments are needed.<sup>11-13</sup>

Gemtuzumab ozogamicin (GO) is an immunotoxin consisting of a potent humanized monoclonal antibody against CD33, and targets CD33 positive cells

which are present in approximately 80-90% of childhood AML.<sup>14,15</sup> Although CD33 has been considered as a specific marker for hematopoietic cells of the myeloid lineage for a long time, this surface marker is also found to be expressed in hepatocytes which may potentially cause some off-target effects.<sup>16</sup>

Treatment with GO in different settings has previously been shown to be of value in pediatric AML. The first international experience of treatment with GO as monotherapy for compassionate use for children with relapsed or refractory AML (n=15) in 2003 suggested the efficacy of this treatment with doses of 4-9 mg/m<sup>2</sup> in up to 3 cycles.<sup>17</sup> A later phase II study showed that treatment with two doses of 7.5 mg/m<sup>2</sup> GO with 14-day intervals in children with advanced AML (refractory, first refractory relapse or ≥second relapse) led to significantly higher survival in patients who received GO compared to patients who did not receive this treatment (3-year probability of overall survival: 27% vs. 0%, respectively; *P*=0.001).<sup>18</sup> In addition to monotherapy, a good response rate was also achieved when GO was administered in combination with cytarabine in 17 children with relapsed or refractory AML [overall complete remission (CR) rate: 53%].<sup>19</sup>

Currently there are at least 9 active clinical trials worldwide investigating the effect of GO in AML (*de novo*, relapsed, or refractory). One of these trials only recruits children and 4 are recruiting children in addition to adults or elderly patients (Online Supplementary Table S1). Of interest, there is one active clinical trial which studies treatment with GO in compassionate use in refractory or relapsed AML (clinicaltrials.gov identifier: 02312037), which recruits children, adult, and elderly groups of patients.<sup>20</sup>

Considering the above-mentioned challenges in the treatment of relapsed and refractory pediatric AML, the infrequency of new treatment options, and in addition, the restricted accessibility of GO, we aimed to identify patients treated with GO as compassionate use in the AML-Berlin-Frankfurt-Münster (AML-BFM) study group and to evaluate the efficacy and safety in this heavily pre-treated group of patients.

## Methods

### Patients

Between January 1995 and March 2014, 2601 children with initial diagnosis at the age of ≤18 years were documented within the AML-BFM study group. Patients or guardians provided written informed consent. The current analysis was performed in accordance with the Declaration of Helsinki and was approved by the local ethics committee. The AML-BFM Study Group centrally reviewed the diagnosis of initial disease or relapse *via* bone marrow morphology and flow cytometry. All patients' records were evaluated retrospectively for the use of GO. Medical reports of the patients treated with GO were reviewed retrospectively for evaluation of treatment outcomes and adverse events (AE).

### Treatment protocols

Before administration of GO, patients were treated based on randomized, phase III studies AML-BFM 93, 98, and 2004 running in 75 centers in Germany, Austria, Switzerland, and the Czech Republic. These treatment protocols have been previously described in detail.<sup>21-23</sup> All studies were performed after the approval by national ethics committees and institutional review boards. The AML-BFM 93 recruited patients between January

1993 and June 1998, and was followed by AML-BFM 98 which was opened in July 1998 and closed in June 2003. Between July 2003 and April 2004, the AML-BFM 98 Interim Study continued treatment of recruited patients with the best arm of the AML-BFM 98 trial. The AML-BFM 04 study was opened in April 2004 and randomization continued until April 2010. After April 2010 and until February 2014, the AML-BFM 2004 Interim Study continued treatment of patients with the experimental arm of the AML-BFM 2004 trial. Second-line treatment of patients included the Relapse AML 2001/01 trial<sup>24</sup> which recruited patients from November 2001 to April 2009 and the International Registry Relapsed AML 2009.<sup>7</sup> Patients included in the current cohort had received intensive treatment and/or HSCT before administration of GO. Considering the first treatment after initial diagnosis as first attempt, and each following treatment block or HSCT as further individual attempts, most of the patients (n=35, 46%) in the total cohort received GO as their 3<sup>rd</sup> treatment attempt (Online Supplementary Figure S1).

### Treatment with GO

During the period of analysis, 217 patients with non-response (NR) and 654 patients with at least one event of relapse were documented (Online Supplementary Figure S2). Within these patient records, 98 (from 39 different centers) were found with a positive history of GO treatment, 10 of which were included in the previously mentioned phase II trial<sup>18</sup> and who were, thus, excluded from the current study. The remaining 88 patients received GO on a compassionate use basis, after failure of their first- and/or second-line treatments and/or when the general condition of patients was so poor that further intensive chemotherapy was not possible. Treatment with GO in these patients was recommended by the centralized study co-ordination office, and finally prescribed by the patients' treating physicians at each local center. Patients or guardians provided written informed consent. GO was provided by International Pharmacy (San Francisco, CA, USA) through Clinigen (London, UK) and, for some patients, by Pfizer (New York, NY, USA) on a compassionate use program. From these 88 patients, 76 had sufficient data to evaluate the outcomes of GO use and were included in this study (Online Supplementary Figure S2). Three of the 76 patients have been previously reported.<sup>17</sup> Grading of toxicity and adverse events was carried out using the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 4.03, revised in June 14, 2010.<sup>25</sup> Records concerning the safety evaluation of GO were not available for 5 patients (Online Supplementary Figure S2).

### Definitions and statistical analysis

Bone marrow (BM) relapse was defined by presence of >5% of leukemic cells in the BM. Relapse events during the first 12 months from diagnosis of AML were considered as early relapse.<sup>3</sup> Complete remission (CR) was defined with the presence of less than 5% blasts in morphological examination of BM in addition to satisfactory hematologic recovery (absolute neutrophil count > 1.0 ×10<sup>9</sup>/L, platelet count >80×10<sup>9</sup>/L in the peripheral blood).<sup>21</sup> Reaching CR without complete blood recovery was defined as incomplete CR (CRi).<sup>21</sup> Response to GO was evaluated in patients who received HSCT after treatment with GO and was defined as CR plus CRi (CR/CRi). Persistence of ≥5% BM leukemic blasts was categorized as NR.

Overall survival (OS) after GO was defined as the time from the first dose until death by any cause or until the last follow up. Probability of OS was calculated according to Kaplan-Meier and compared by log-rank test. *P*<0.05 was considered significant. Data were analyzed using SAS (Statistical Analysis System Version 9.4; SAS Institute, Cary, NC, USA). Data acquisition was stopped on 1<sup>st</sup> June 2017.

## Results

### Patients' characteristics and treatment with GO

The majority of patients were initially diagnosed with AML with the French-American-British (FAB) classification of M4 (without atypical eosinophils) or M5 (n=29,

39%) and 13 (17%) patients had white blood cell (WBC) counts higher than  $100 \times 10^9/L$  at the time of their diagnosis. In total, 67 (88%) patients had high-risk AML as defined by morphology, cytogenetics and response to treatment, retrospectively (Table 1). Most of the patients (n=43, 56%) had been previously treated with liposomal

**Table 1.** Patients' characteristics at initial diagnosis.

Feature	n	%
Total number of patients	76	100
Age, years		
Median (range)	9.3 (1 months - 17 years)	
Categories		
0-2	15	20
3-10	26	34
11-18	35	46
Sex		
Female	33	43
Male	43	57
FAB Classification		
M0	6	8
M1/M2	25	33
M3	1	1
M4Eo-/M5	29	39
M6	1	1
M7	7	9
AML-not classified	7	9
WBC count at diagnosis		
Median $\times 10^9/L$ (range)	14.8 (0.39 - 324)	
Patients with WBC $\leq 100 \times 10^9/L$	62	82
Patients with WBC $> 100 \times 10^9/L$	13	17
No data	1	1
Previous treatments		
Pre-treatment with FLA/G	12	16
Pre-treatment with FLA/G+DX (+/- FLA/G) <sup>a</sup>	43	56
Pre-treatment without FLA/G+DX or FLA/G	21	28
HSCT prior to GO treatment		
Yes	14	18
No	62	82
Disease status prior to GO		
Refractory <i>de novo</i> AML <sup>b</sup>	10	13
<i>De novo</i> AML- refractory to 1 <sup>st</sup> early relapse <sup>c</sup>	41	54
<i>De novo</i> AML- refractory to 1 <sup>st</sup> late relapse <sup>c</sup>	10	13
<i>De novo</i> AML- refractory to 2 <sup>nd</sup> relapse <sup>c</sup>	8	11
<i>De novo</i> AML- refractory to 3 <sup>rd</sup> relapse <sup>c</sup>	2	3
Secondary AML- refractory to 1 <sup>st</sup> /2 <sup>nd</sup> relapse <sup>c</sup>	5	6
Risk group <sup>d</sup>		
Standard risk	9	12
High risk	67	88

n: number; FAB classification: French-American-British classification; M4Eo: AML M4 subtype without the presence of atypical eosinophils; AML: acute myeloid leukemia; HSCT: hematopoietic stem cell transplantation; FLA/G: fludarabine, cytarabine with or without granulocyte colony stimulating factor; DX: liposomal daunorubicin; GO: gemtuzumab ozogamicin; WBC: white blood cell. <sup>a</sup>Some of the patients in this category received FLA/G in addition to FLA/G+DX. <sup>b</sup>GO was administered after failure of first-line treatment(s). <sup>c</sup>GO was administered after failure of treatment for the relapse episode. <sup>d</sup>Standard risk indicates FAB M1/2 with Auer rods, FAB M4 with atypical eosinophils (M4Eo), FAB M3 and/or favorable cytogenetics, such as t(8;21) and/or AML1-ETO, t(15;17), and/or PML-RARA and inv(16) or t(16;16) and/or CBFβ/MYH1, if there was no persistence of BM blasts ( $\geq 5\%$ ) on day 15. FLT3-ITD positivity was not considered. All others were classified as high-risk patients.

daunorubicin and fludarabine in addition to cytarabine with or without granulocyte colony stimulating factor (FLA/G+DX). Fourteen (18%) patients received HSCT prior to treatment with GO (Table 1). GO was administered after failure of treatment attempts at different time points of therapy and most (n=41, 54%) of the patients were treated with GO after failure of treatment(s) for first-early relapse of *de novo* AML (*de novo* AML, refractory to

first early relapse) (Table 1). GO was equally prescribed as either monotherapy (n=36, 47%) or in combination with cytarabine (n=36, 47%), and the remaining patients (n=4, 6%) received GO in combination with other agents (Table 2). Most patients (n=48, 63%) received one cycle of GO as monotherapy or combination therapy (Table 2). GO was frequently administered at doses of 3 mg/m<sup>2</sup> or lower (n=37, 49%) (Table 2) and most of these patients received

**Table 2.** Details and outcomes of treatment with gemtuzumab ozogamicin.

Disease status prior to GO	Refractory <i>de novo</i> AML	<i>De novo</i> AML, refractory to 1 <sup>st</sup> early relapse	<i>De novo</i> AML, refractory to 1 <sup>st</sup> late relapse	<i>De novo</i> AML, refractory to ≥2 <sup>nd</sup> relapses/secondary AML	Total cohort
Number (%)	10 (100)	41 (100)	10 (100)	15 (100)	76 (100)
Age, median (range) at initial diagnosis (years)	10 (2 months -14)	7.5 (4 months -17)	12.6 (8 months - 16)	10.3 (1 month -16)	9.3 (1 month -17)
at treatment with GO (years)	10.3 (7 months -15)	8.1 (1 - 18)	15.7 (2 - 20)	12.2 (9 months -16)	10.4 (7 months - 20)
Treatment with GO					
monotherapy	4 (40)	17 (41)	3 (30)	12 (80)	36 (47)
GO + cytarabine	6 (60)	20 (49)	7 (70)	3 (20)	36 (47)
other	0 (0)	4 (10)	0 (0)	0 (0)	4 (6)
Number of cycles					
one	6 (60)	27 (66)	7 (70)	8 (53)	48 (63)
two	4 (40)	10 (24)	3 (30)	7 (47)	24 (32)
three	0 (0)	3 (8)	0 (0)	0 (0)	3 (4)
four	0 (0)	1 (2)	0 (0)	0 (0)	1 (1)
GO dosage at the first cycle					
≤ 3 mg/m <sup>2</sup>	6 (60)	20 (49)	7 (70)	4 (27)	37 (49)
3.1 – 5.4 mg/m <sup>2</sup>	2 (20)	5 (12)	0 (0)	1 (7)	8 (10)
5.5 - 7.5 mg/m <sup>2</sup>	2 (20)	10 (24)	1 (10)	8 (53)	21 (28)
> 7.5 mg/m <sup>2</sup>	0 (0)	4 (10)	2 (20)	2 (13)	8 (10)
n.d.	0 (0)	2 (5)	0 (0)	0 (0)	2 (3)
HSCT after GO treatment					
Yes	8 (80)	28 (68)	8 (80)	5 (33)	49 (64)
1 <sup>st</sup> HSCT	8	27	8	3	46
2 <sup>nd</sup> HSCT	0	1	0	2	3
No	2 (20)	13 (32)	2 (20)	10 (67)	27 (36)
Extra treatment prior to HSCT <sup>a</sup>					
Chemotherapy	2 (25)	9 (32)	2 (25)	1 (20)	14 (28)
No-treatment	6 (75)	16 (57)	5 (63)	2 (40)	29 (60)
Other	0 (0)	1 (4)	1 (12)	1 (20)	3 (6)
n.d.	0 (0)	2 (7)	0 (0)	1 (20)	3 (6)
Bone marrow response prior to HSCT <sup>a</sup>					
CR	2 (25)	1 (4)	1 (12)	1 (20)	5 (10)
CRi	2 (25)	13 (46)	2 (25)	3 (60)	20 (41)
NR	3 (38)	12 (43)	3 (37)	1 (20)	19 (39)
n.d.	1 (12)	2 (7)	2 (26)	0 (0)	5 (10)
Patient status					
Alive	3 (30)	5 (12)	5 (50)	2 (13)	15 (20)
Deceased	7 (70)	36 (88)	5 (50)	13 (87)	61 (80)

GO: gemtuzumab ozogamicin; n.d.: no data; HSCT: hematopoietic stem cell transplantation; CR: complete remission; CRi: complete remission with incomplete hematologic recovery; NR: non-response. <sup>a</sup>Percentages in this category were calculated only for patients who received HSCT.



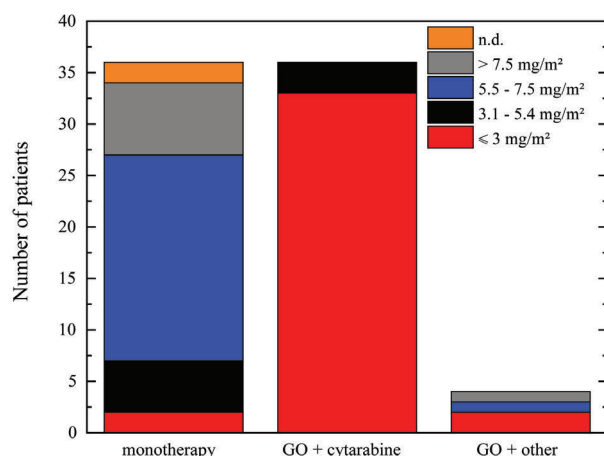
GO in combination with cytarabine or other agents following the failure of treatment after first early relapse ( $n=20$  of 37, 54%) (*data not shown*). When GO was administered as monotherapy, higher doses were used (Figure 1).

### Safety and toxicities

Among 71 patients who were evaluated for AEs during the first cycle of treatment, most common AEs were grade 3/4 infections or febrile neutropenia (61 events in 49 patients, 69%) (Table 3). Two patients suffered from sepsis and were successfully managed with supportive and anti-bacterial therapy. Gastrointestinal (GI) symptoms (11 grade 1/2 events in 11 patients and 4 grade 3/4 events in 4 patients) and immunologic reactions such as infusion-related fever, chills, or hypotension (7 grade-1/2 events in 7 patients and 5 grade 3/4 events in 5 patients) were most frequently observed after infections. Due to interference with other treatments, GI events could not be assigned to GO with certainty.

Among grade 3 and 4 AEs, febrile neutropenia was most frequently observed in both groups of patients with monotherapy and combination therapy as well as in all different groups of patients independently of their previous treatments (FLA/G or FLA/G+DX or patients with HSCT prior to GO) (*data not shown*). The majority (4 of 5) of patients with severe infusion-related immunological reactions to GO treatment, had previously received GO as monotherapy with higher doses compared to patients with combination therapy (*data not shown*).

Veno-occlusive disease (VOD) occurred in 3 patients who received GO as monotherapy (one cycle) at doses of 6, 7.5, and 9 mg/m<sup>2</sup>. All 3 patients had FLA/G+DX as treatment before GO, and received defibrotide as prophylaxis against VOD. Two of these patients were treated with HSCT prior to treatment with GO (6 or 7.5 mg/m<sup>2</sup>). The patient treated with 6 mg/m<sup>2</sup> GO had a previous history of VOD and developed a GO-related VOD before the scheduled HSCT that was successfully treated without late effects or events. The other 2 patients could not receive HSCT after treatment with GO and both died. Cause of death in one of them was disease (leukemic) pro-

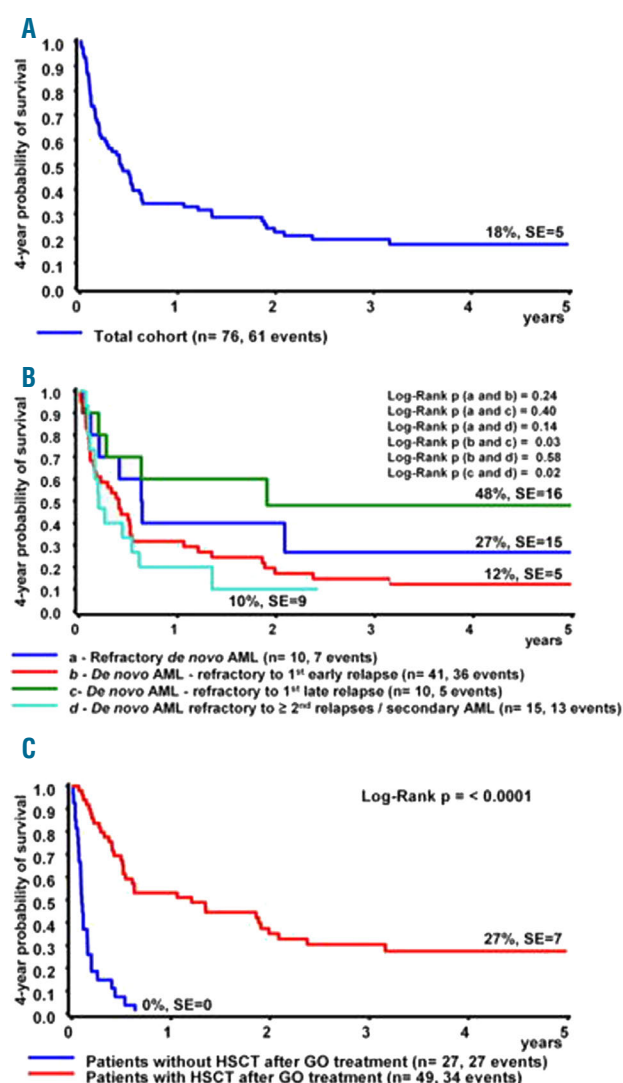


**Figure 1. Gemtuzumab ozogamicin (GO) dosage.** GO dosage based on administration with cytarabine or other agents. AML: acute myeloid leukemia; n.d.: no data.

gression, and the other patient experienced exacerbation of a pre-existing cardiomyopathy leading to death 24 days after GO treatment. In addition, treatment with GO resulted in exacerbation of previous symptoms in 2 patients (pulmonary aspergillosis infection or gastrointestinal toxicity) and, due to overlap with other treatments and HSCT, the consequences of these AEs could not be exclusively correlated to GO (Table 3). Similarly, the consequences of different AEs in one patient with GO-related cytokine syndrome and pre-existing respiratory distress could not be distinguished from each other (Table 3). The respiratory distress in this patient was related to an aplasia-associated pneumonia which was caused by treatments before GO.

### Outcome

With a median follow up time of 4.3 years (range: 1-5 years), the probability of 4-year OS after treatment with



**Figure 2. Survival after gemtuzumab ozogamicin (GO).** (A) 4-year probability of overall survival (OS) in all patients. (B) 4-year probability of OS in different disease statuses. (C) 4-year probability of OS based on administration of hematopoietic stem cell transplantation (HSCT). pOS: probability of OS; n: number of patients; AML: acute myeloid leukemia; SE: Standard Error.

GO was 18±5% in the total cohort (Figure 2A). A comparison of disease status prior to treatment with GO between patient groups showed that patients who received GO after *de novo* AML refractory to first late relapse had a probability of 4-year OS of 48±16%; this is significantly higher than in patients with *de novo* AML refractory to first early relapse (probability of 4-year OS: 12±5%;  $P=0.03$ ) and patients with *de novo* AML refractory to second or more relapses/secondary AML (probability of 4-year OS: 10±9%;  $P=0.02$ ) (Figure 2B). Based on the current retrospective analysis, factors such as number of cycles and administration of GO as monotherapy or in combination with cytarabine or other agents did not have any significant influence on survival (Online Supplementary Figure S3A-C).

In the total cohort of patients, 27 (36%) did not receive HSCT after treatment with GO (Table 2) and none of these patients survived (Figure 2C). Of note, most (10 of 15, 67%) patients with *de novo* AML refractory to second or more relapses/secondary AML, did not receive an HSCT after GO treatment; this is higher than in other groups of patients (Table 2).

From the total group of 76 patients, 49 (64%) received HSCT after GO treatment and their probability of 4-year OS was 27±7%. Thirty (61%) patients with HSCT received GO in combination with cytarabine (*data not shown*). HSCT after GO was the first HSCT for most of the patients ( $n=46$ , 94%), and mean time to transplantation after GO treatment was 41±30 days (range 11-135 days). The probability of 4-year OS in patients who received HSCT early during the first three weeks after GO treatment was 9±9% and patients who received HSCT between three and six weeks after GO administration had a 4-year OS of 40±11% ( $P=0.06$ ) (Online Supplementary Figure S3D).

Previous treatment with or without FLA/G+DX before GO administration had no influence on the percentage of

HSCT achieved after treatment with GO (FLA/G+DX group: 29 of 43, 67%; no FLA/G+DX or FL/A group: 14 of 21, 67%) (*data not shown*). However, among transplanted patients, fewer patients with previous FLA/G+DX (7 of 29, 24%) survived compared to the patients without FLA/G+DX or FL/A (6 of 14, 42%) (*data not shown*). In addition, one patient with *de novo* AML-M3 refractory to first late relapse is alive after treatment with GO followed by additional chemotherapy and HSCT.

Among 49 patients with subsequent HSCT, 25 (51%) reached CR/CRi before HSCT (Table 2). Out of 19 patients with no response to GO before HSCT, 8 (42%) received additional chemotherapy before HSCT and 3 of them survived. The remaining 11 (58%) patients with no response, received HSCT with no further chemotherapy and 2 of these patients survived (*data not shown*).

## Discussion

The major goal in the treatment of relapsed/refractory pediatric AML is to develop new therapeutic options to achieve complete remission and proceed to HSCT thereafter.<sup>26</sup> Considering the previous history of intensive chemotherapy and/or previous HSCT in these patients, novel treatment options should be efficient, but must also have acceptable toxicity profiles. However, the introduction of new drugs is challenging. Due to increasing regulatory challenges and requirements, as well as limited accessibility of drugs such as GO, it is more and more time consuming to open phase III trials. Hence a follow-up trial, that has been planned since 2009 after the end of the phase II trial,<sup>18</sup> was not opened until September 2016 and recruited the first patient in August 2017 (EudraCT n.: 2010-018980-41).

In the current analysis, we have evaluated the use of GO

**Table 3. Non-hematologic adverse events in 71 patients after the first treatment cycle with gemtuzumab ozogamicin.**

AE severity/ consequence	All grades <sup>a</sup>	Grades 1/2				Grades 3 and 4							
	Total AEs,	reversible		reversible		prolonged reversible		progressive		fatal		n.a.	
	All outcomes n (%)	AEs	Patients	AEs	Patients	AEs	Patients	AEs	Patients	AEs	Patients	AEs	Patients
Gastrointestinal	15 (15)	11	11	4	4	0	0	0	0	0	0	0	0
Pain	4 (4)	1	1	3	3	0	0	0	0	0	0	0	0
Infection or fever in neutropenia	61 (60)	0	0	58	46	2	2	1 <sup>b</sup>	1	0	0	0	0
VOD	3 (3)	0	0	1	1	1	1	0	0	1 <sup>c</sup>	1	0	0
Infusion-related immunological reactions	12 (12)	7	7	4	4	0	0	0	0	0	0	1 <sup>d</sup>	1
Exacerbation of the previous conditions	2 (2)	0	0	0	0	0	0	0	0	0	0	2 <sup>e</sup>	2
Constitutional symptoms	2 (2)	2	2	0	0	0	0	0	0	0	0	0	0
Metabolic/laboratory	2 (2)	2	2	0	0	0	0	0	0	0	0	0	0
Total, n <sup>f</sup> (%)	101 (100)	23 (23)		70 (69)		3 (3)		1 (1)		1 (1)		3 (3)	

AE: adverse events; n: number; n.a.: not applicable; VOD: veno-occlusive disease. <sup>a</sup>Grading of toxicity and adverse events based on the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 4.03, revised June 14, 2010. <sup>b</sup>Progressive fever partly resulted from limiting its treatment due to joint decision to terminate the "active" therapy of the patient. <sup>c</sup>GO-related VOD led to exacerbation of a pre-existing cardiomyopathy causing death 24 days after the treatment with GO. <sup>d</sup>Treatment with GO resulted in occurrence of cytokine syndrome in a patient with pre-existing respiratory distress due to aplasia-associated pneumonia. Following a decrease in level of consciousness, this patient died five days after occurrence of the cytokine syndrome. <sup>e</sup>Treatment with GO resulted in worsening of previously present pulmonary aspergillosis infection or in worsening of previously observed gastrointestinal toxicity. <sup>f</sup>All adverse events in 71 patients were evaluated.



on a compassionate use basis in a cohort of children with refractory *de novo* AML and *de novo* or secondary AML refractory to relapse therapy. The use of GO in heavily pre-treated patients translated into a substantial rate of subsequent HSCT of 64% and a probability of 4-year OS of 27% in these patients. The safety profile in our cohort was tolerable, which may allow the use of GO in patients with a history of intensive therapies.

We have identified 88 and analyzed 76 children with relapsed/refractory AML who were treated with GO on a compassionate use basis. Compared to other studies which evaluated the outcomes of treatment with GO in patients with relapsed/refractory AML either on a compassionate use basis (Zwaan *et al.*, 15 patients;<sup>17</sup> Brethon *et al.*, 17 patients)<sup>19</sup> or with other treatment strategies (29 patients with relapsed/refractory AML in an open-label, dose-escalation study;<sup>27</sup> 45 children with relapsed/refractory AML included in the AAML00P2 randomized clinical trial;<sup>28</sup> 30 children with advanced relapsed/refractory AML in an investigator-initiated phase II study)<sup>18</sup> the current cohort contains the largest number of patients.

With GO doses of 2.5-10 mg/m<sup>2</sup>, 64% of the patients included in our cohort subsequently received HSCT, and 51% of these patients reached complete remission with or without hematologic recovery before receiving HSCT. In 2005, Arceci *et al.* studied the effect of treatment with GO as a single agent with doses of 6-9 mg/m<sup>2</sup> (2 doses, 2-week intervals) in 10 children with refractory AML and 19 with relapsed AML.<sup>27</sup> They defined response to GO as achieving CR (presence of  $\leq 5\%$  blasts in BM with full hematologic recovery: hemoglobin level  $\geq 9$  g/dL, absolute neutrophil count  $\geq 1.5 \times 10^9$ /L, and platelet count  $\geq 100 \times 10^9$ /L) and showed a response rate of 30% and 26% in patients with refractory and relapsed AML, respectively, accompanied by acceptable safety profiles.<sup>27</sup> In addition, in a study by Brethon *et al.*, in 2006, outcome of monotherapy with GO on a compassionate basis (single dose: 3-9 mg/m<sup>2</sup>) in children with refractory (3 patients) and relapsed (9 patients) AML was investigated.<sup>29</sup> The results of this study showed a CR (plus CR without hematologic recovery) rate of 25% in patients with subsequent HSCT.<sup>29</sup> Of note, a subsequent study by the same group in 2008 showed that the response rate was higher (overall CR: 53%) when GO was administered in combination with cytarabine in children with relapsed or refractory AML.<sup>19</sup> We could not confirm the benefit of this combination treatment in our cohort since combination of GO with cytarabine alone, or with cytarabine along with other agents or combination of GO with vincristine had no survival advantage compared to single agent therapy.

Myelosuppression accompanied by fever was the most

common GO-related AE in our patients (49 events in 58% of patients) (*data not shown*) that appeared to be independent of GO dosage, combination/monotherapy, previous treatments, or disease status prior to GO. Rates of myelosuppression by GO were previously reported at a range of 25-100% in different studies.<sup>19,28,29</sup>

In our cohort, the rate of VOD after treatment with GO was 4%. Frequency of VOD related to monotherapy or combination GO therapy in children has been previously reported with a wide range across different studies from no events<sup>29,30</sup> to 10-24%.<sup>27,28,31</sup> However, taken together, the results of previous studies show that the incidence of VOD is directly associated with the absolute single dose of GO, which is higher when it is administered as monotherapy.<sup>32</sup> These findings are in accordance with the results of the current cohort. Considering the suggested increased risk of VOD in cases of monotherapy with GO, and the lack of a survival advantage between monotherapy and combination therapy in our current cohort, administration of GO in combination with other agents, such as cytarabine, may be useful to prevent this complication.

It should be noted that the current analysis is limited by its retrospective study design and especially by the lack of a control group. These restrictions have hindered our efforts to identify the contribution of compassionate treatment with GO as a single factor to the outcomes of the current study.

In conclusion, we have shown in a large cohort of patients with relapsed/refractory *de novo* and secondary AML, with a history of very intensive treatments including chemotherapy and/or HSCT, that administration of GO on a compassionate use basis was frequently considered. The study provides evidence that GO can enable a subsequent blast reduction that allowed HSCT in these patients and survival without imposing major adverse events. Since addition of GO showed the potential to improve treatment outcomes in the current cohort of patients with relapsed/refractory pediatric AML, the role of GO in these patient groups should ideally be proven in large prospective randomized clinical trials. Therefore, we have now included GO as front-line treatment of relapsed/refractory pediatric AML in our ongoing phase III multicenter clinical trial (EudraCT number: 2010-018980-41, recruiting since August 2017).

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